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Molecular systematics of Characodon: Phylogeny based on a nuclear locus

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Molecular systematics of *Characodon*: Phylogeny based on a nuclear locus

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by

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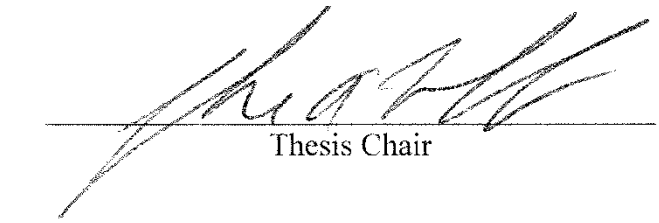

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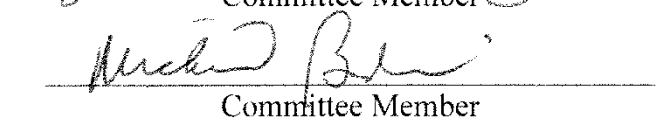

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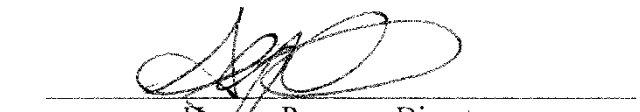

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Abstract

Characodon is a genus of livebearing fishes whose two extant species (*C. lateralis* and *C. audax*) inhabit localities along the Río Mezquital of Durango, Mexico. This lineage of Goodeidae (Cyprinodontiformes) is critical to study because of its biogeographic and phylogenetic positions within the group, and both species are of conservation concern. A recent mitochondrial DNA analysis contradicts the published taxonomy, and suggests that *Characodon* has diverged into northern and southern populations. This, coupled with the observation that the morphological characteristics used in the original species descriptions might be flawed, has led me to study the phylogenetic relationships among populations using a third kind of evidence, nuclear DNA. The flanking regions of a microsatellite locus, amplified and sequenced using standard protocols, were compared for 20 specimens representing one population of *C. audax* and six populations of *C. lateralis*. Four non-*Characodon* outgroups were used to root the phylogeny. The DNA sequences of *C. audax* were found to be identical to those of the northern *C. lateralis*, and the southern *C. lateralis* were recovered as a clade that excluded the northern populations, consistent with the mitochondrial analysis. A relative dearth of sequence variation means this finding should be evaluated cautiously, but it appears that morphological, mitochondrial, and nuclear evidence are in agreement that *Characodon* diversity needs to be redescribed. This project was supported by the Biology Department and Honors Program of the University of North Georgia.

Introduction

Fishes within the clade (group) Goodeidae occur on the Mexican highlands (Figure 1) and in the southwestern United States (Webb *et al.* 2004; Doadrio and Domínguez, 2003). This diverse group comprises 18 genera with about 36 small to medium-sized, livebearing species that display varying levels of sexual dimorphism (Doadrio and Domínguez, 2003; Hamill *et al*., 2007). Many goodeids are in need of conservation, so the need to study them goes beyond the academic. Many species are threatened, endangered, or facing extinction. Populations of these fishes in general have been shrinking over the last 100 years, because their aquatic ecosystems have been disturbed by human development (Figure 2). Changes in water quality from pollution, soil erosion, and eutrophication affect these fishes (Domínguez-Domínguez *et al*., 2006). *Characodon* is an imperiled group of Goodeidae. This genus occurs north of the main clades of Mexican goodeids and is made up of multiple populations that occur in small-volume systems adjacent to the Río Mezquital (Figure 1). To address this crisis, it is necessary to identify species and assess the amount of genetic diversity within populations so that scientists can determine how best to focus conservation efforts.

Recent morphological and phylogenetic evidence suggest that recognized species of *Characodon* may not be valid (Tiedemann, 2009; Howell *et al.,* 2008). The purpose of this study is to analyze the diversity of the group. Tiedemann (2009) questioned the morphological characters used to define *Characodon audax*, and Howell *et al*. (2008), using mitochondrial DNA, showed that this taxon's relationship to *Characodon lateralis* is dubious. Here, nuclear DNA will be examined to clarify the relationship between *C. lateralis* and *C. audax*.

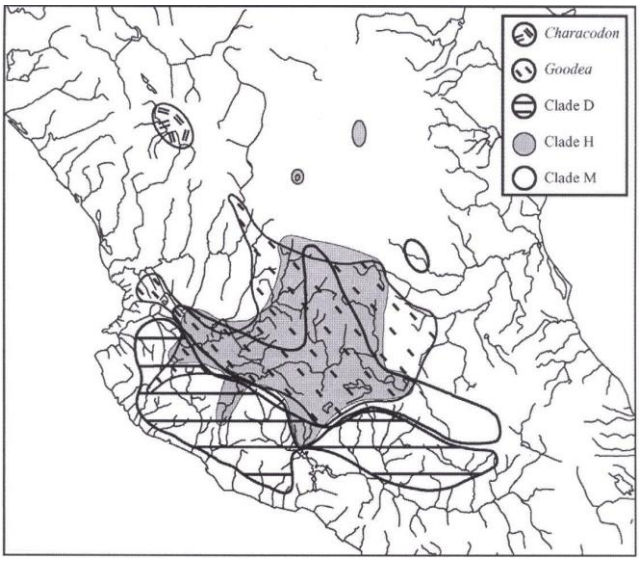

Figure 1. The distribution of Goodeidae in central Mexico. Note the hydrological networks in addition to the placement of *Characodon*. The figure was adapted from Webb *et al.* 2004, and information on the specific clades is provided in fig. 5 of that paper.

History

Characodon is important because its extant taxa are endangered. *Characodon garmani*, first reported in 1895, became extinct between 1900 and 1953 (Smith and Miller, 1986). *C. audax,* one of the species this study is concerned with, only resides in a small spring along the Río Mezquital, and is consequently endangered (Dominguez-Dominguez *et al*., 2006). *C. lateralis* is also threatened. Its population has been in decline and is now only found along the Río Mezquital (Smith and Miller 1986). *Characodon* also has significant phylogenetic and biogeographic positions within the subfamily Goodeinae, for it last shared a common ancestor with the rest of the goodeines about 14.9 million years ago (Webb *et al*., 2004). Webb *et al*. hypothesized that the Río Mezquital was once connected to the rest of central Mexico about 14.9 million years ago, so studying *Characodon* helps researchers understand the life history and development of its subfamily (2004). Conservation efforts are predicated on knowing the diversity of *Characodon,* and previous research has questioned the validity of *C. audax* (Howell *et al*., 2008; Tiedemann 2009).

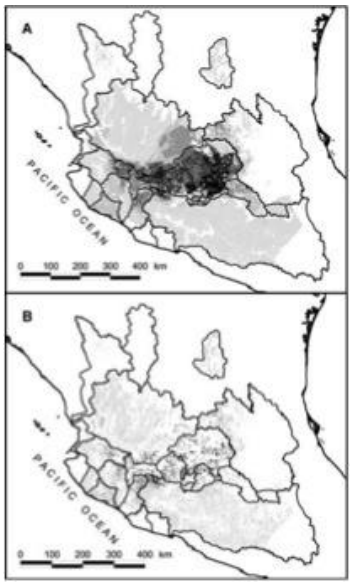

Figure 2. A map of goodeine species richness obtained through niche modeling. "A" represents potential goodeine species diversity based on resources available. "B" represents current species diversity. Darker areas represent higher species diversity (maximum 8). Adapted from Dominguez-Dominguez *et al.*, 2006.

Morphology

Morphological analysis has classically been used to distinguish and systematize goodeid taxa. Hubbs and Turner (1939) classified the goodeids based upon one of the most prominent traits, their viviparity (ability to bear live young). The structures most commonly utilized in comparisons have been the ovaries of females and the trophotaeniae (rectal processes comparable to placentas) of embryos. Comparison of the reproductive anatomy among taxa has been used to define major groups, but more detailed measurements of the body have been used to differentiate species (Figure 3) (Smith and Miller, 1986). It should be noted that the relationships suggested by Hubbs and Turner (1939) have been contradicted by later phylogenetic analyses of mitochondrial DNA loci (Doadrio and Domínguez, 2003; Webb *et al.*, 2004).

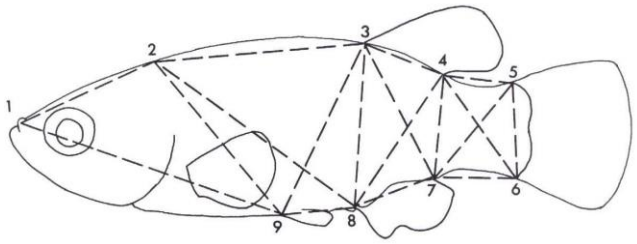

Figure 3. Morphology can be assessed with measurements of various distances across the fish's body as well as by counting the number of scales along the body. Image adapted from Smith and Miller (1986).

C. lateralis was described in 1865 using broad, nearly useless, morphological characteristics (Garman). *C. audax* was later described as distinct from *C. lateralis* based upon more specific morphological features (Smith and Miller, 1986). To morphologically categorize the species of *Characodon*, Smith and Miller (1986) measured straight-line segments between discrete anatomical points on the fishes' bodies (Figure 3 above). They recognized three species of *Characodon* using 3 features for diagnosis: *C. lateralis*, *C. audax* (newly discovered at the time), and *C. garmani*. *C. garmani* went extinct before they could conduct their study, so they had to rely on measurements of a preserved female, which was collected in 1895. They analyzed *C. lateralis*, *C. audax,* and *C. garmani* and determined that they were all valid species (Smith and Miller 1986). More recently, Tiedemann (2009) analyzed *C. lateralis* and *C. audax* and concluded that the analysis by Smith and Miller was likely flawed. For example, one feature Smith and Miller used to identify *C. audax* was pelvic fin length. When Tiedemann reexamined this feature (and the others) from the same collections, she found that there was much more variation than Smith and Miller originally documented. Tiedemann found *C. lateralis* and *C. audax* to be morphologically indistinguishable from one another (Tiedemann 2009).

Molecular Systematics

Phylogenetic trees portray evolutionary relationships since a most recent common ancestor. Many systematists now employ mitochondrial DNA (mtDNA) sequences to study interrelationships as it mutates at a faster rate than nuclear DNA and it does not recombine (Wilson *et al*., 1985). Much work has gone into determining the phylogeny (evolutionary relationships) of Goodeidae by sequencing various mitochondrial loci. Sequence data has been used to estimate divergence dates among groups and can provide a detailed phylogeny (Webb *et al*., 2004; Doadrio and Domínguez, 2003). For example, Webb *et al*. used 36 species of all 18 goodeid genera and 8 additional cyprinodontoids with segments of the mitochondrial control region and cytochrome oxidase I (2004). Their parsimony-based tree (Figure 4) was significantly different from the relationships proposed by Hubbs and Turner (1939).

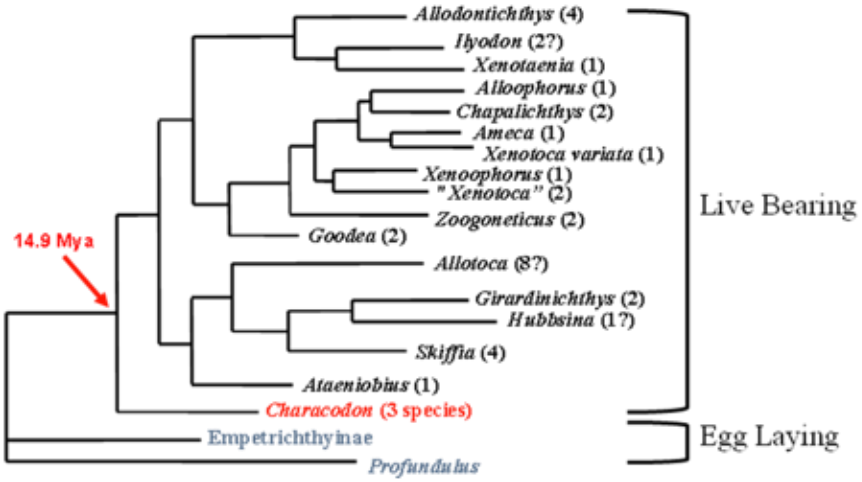

Figure 4. A modified phylogentic tree of the Goodeidae from Webb *et al.* (2004) based on the mitochondrial c oxidase subunit I gene. Adapted from Tiedemann and Webb (2009).

The goal of this experiment is to obtain a most-parsimonious phylogenetic tree of *Characodon* populations in hopes of evaluating the validity of *C. audax* and *C. lateralis*. There are two possibilities regarding that tree. Either the *Characodon* species will be reciprocally monophyletic (they all share a common ancestor and its descendants), or one or both of them

will be paraphyletic (they do not contain all of the descendants) (Figure 5). A monophyletic outcome would support *C. audax* as valid, whereas a paraphyletic outcome would suggest that *C. audax* may not truly be a separate species (Tiedemann 2009).

Howell *et al*. (2008) sequenced 420 nucleotides of the mitochondrial control region to compare *C. lateralis* and *C. audax* with a few outgroups. What they found was just as surprising as Tiedemann's result; their analysis revealed a paraphyletic relationship. Their tree is shown in Figure 6. Another tree has to be inferred because mitochondrial loci, while they do mutate faster than nuclear loci, could pose a risk for dispersal (Wilson *et al*., 1985). Mitochondria are typically inherited through the female (Wilson *et al*., 1985), so a single female *C. lateralis* could potentially pass her mitochondria onto all of the *C. audax* through several generations of breeding if there were even one case of dispersal. It would be ideal to test Howell *et al*.'s hypothesis with nuclear evidence to ensure that the *C. audax* mitochondrial genome is not just a *C. lateralis* mitochondria.

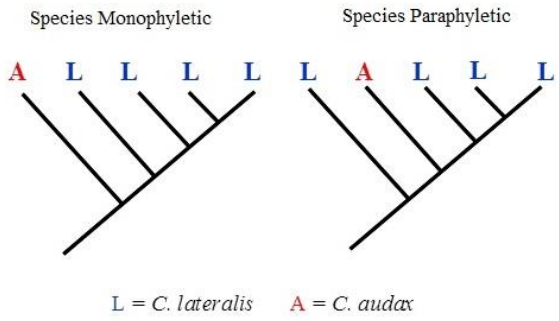

Figure 5. The two possible outcomes from comparing *C. lateralis* and *C. audax*. The right tree represents a paraphyletic relationship. Howell *et al.* had a paraphyletic tree, which is represented by the tree on the right. Figure modified from Tiedemann (2009).

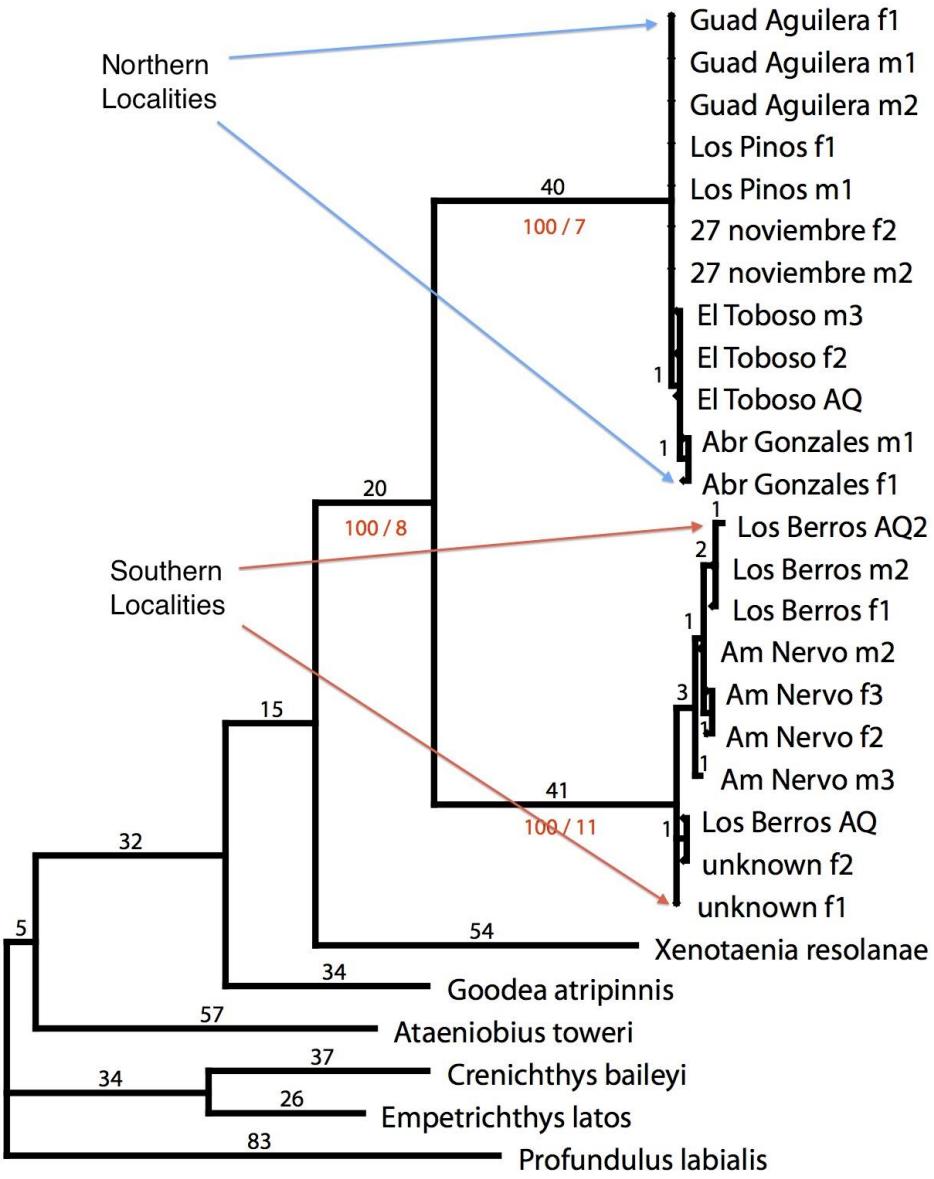

Figure 6. The paraphyletic tree produced by Howell *et al.* (2008). El Toboso represents *C. audax*, which is grouped with the northern populations. Each number above the line represents the number of nucleotide changes on that branch.

Based on the morphological and mitochondrial analyses of *Characodon*, it is evident that there is still more work to be done. Nuclear DNA provides another data set for *Characodon* systematics outside of morphology or mtDNA. Many studies have used microsatellite loci for assessing genetic diversity (Bailey *et al*., 2007; Boto and Doadrio, 2003; Hamill *et al*., 2007), for

these nuclear loci evolve quite rapidly (Oliveira *et al*., 2006). These studies have determined the oligonucleotide primers for microsatellites (Boto and Doadrio, 2003; Bailey *et al.*, 2007; Hamill *et al*., 2007). Microsatellites are non-coding DNA loci that have repeating motifs 1 to 6 nucleotides long. The mutation rate in microsatellite loci is 10^{-2} to 10^{-6} nucleotides per generation, much higher than that of the genome on average (Oliveira *et al*., 2006). However for the purposes of this study, the repeat regions would not be ideal to compare because their variation is quantitative. The accompanying flanking regions (the areas around the repeat motifs), on the other hand, mutate at the same rate as the genome. Here, the current study will test the two existing hypotheses (Smith and Miller, 1986; Howell *et al*., 2008) with data from a phylogenetic tree produced from the flanking regions of a microsatellite locus.

Methods

This study used specimens of *Characodon* previously collected from seven localities adjacent to the Río Mezquital of Durango, Mexico. Six collections are of *C. lateralis* specimens from northern and southern localities (Figure 7). The last collection is of *C. audax*, whose only population is located in an isolated spring adjacent to the northern populations of *C. lateralis* (Smith and Miller, 1986). Additionally, outgroup taxa (controls) including the goodeids *Xenotaenia resolanae*, *Goodea atripinnis, Crenichthys baileyi*, *Ataeniobius toweri* and *Empetrichthys latos* and non-goodeid *Profundulus labialis* were used to root the tree (as controls).

Genomic DNA was extracted with a DNeasy Blood and Tissue kit (Qiagen, Inc). The microsatellite locus ZT1.9 (500 bp long) was amplified using the F and R primers of Boto and Doadrio (2003). The samples were amplified through 50 µL polymerase chain reactions (PCR) using GoTaq® Green Master Mix (Promega, Inc). Standard PCR's included 25 µL of master

mix, 17 µL of nuclease free water, 4 µL of template, 2 µL of forward (F) primer, and 2 µL of reverse (R) primer. The PCR products were separated with 1.5% agarose gel electrophoresis at 60V for 5 minutes and 100V for 40 minutes (Sambrook and Russell, 2001), with the desired product being extracted out of the gel using a QIAquick gel extraction kit (Qiagen, Inc.). After checking extracted DNA concentrations with a spectrophotometer (25 ng/µL of DNA was needed for sequencing), products were sent to the Georgia Genomics Facility of the University of Georgia for Sanger termination reactions and autosequencing using a 3730xl DNA Analyzer (Applied Biosystems).

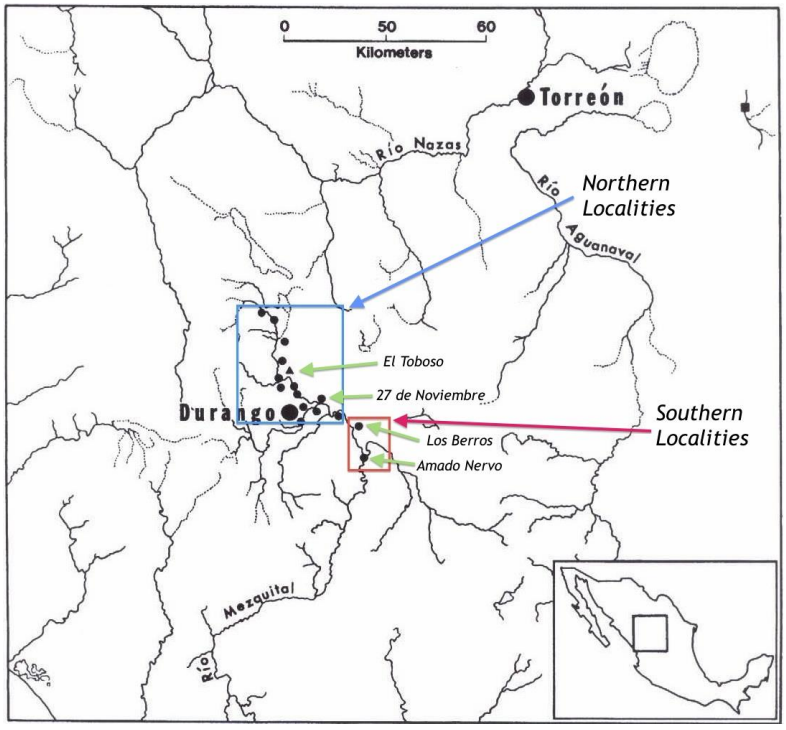

Figure 7. A modified map of the localities of *Characodon*, adapted from Smith and Miller (1986). The triangle within the northern localities represents the isolated spring that contains *C. audax*. A waterfall (El Salto) separates the northern and southern localities. Not all northern and southern localities used in this experiment are portrayed on this map.

The forward and reverse sequences obtained from the Georgia Genomics Facility were checked by hand using BioEdit (Hall 2013) and the sequences of the different individuals were aligned using the CLUSTALW (Thompson *et al*. 1994) function of Biology Workbench (Maer *et al*. 1999) (Table 1). Extensive quantitative variation among *Characodon* samples made alignment difficult, so the repeat region (microsatellite) within the locus was removed for analysis. The CLUSTAL W alignment was used to produce a most-parsimonious phylogenetic tree, and PAUP was programmed to do a heuristic search with 20 random, stepwise addition replicates, using parsimony and outgroup rooting. The relationships of the *Characodon* populations were assessed using consistency, rescaled consistency, and homoplasy indices. In addition, the tree was compared to the published taxonomy and Howell *et al*.'s tree (Figure 6) (2008).

Two specimens from the Guadalupe Aguilera locality showed evidence of contamination, so they were not included in the tree. *Ataeniobius toweri*, one of the outgroups, did not yield any readable sequence. Another outgroup, *Profundulus labialis* did yield sequence, but it was improperly placed in the tree. *P. labialis* was removed for clarity's sake.

	Northern *Characodon*	Southern *Characodon*	*Profundulus labialis*	*Xenotaenia resolanae*	*Goodea atripinnis*	*Empetrichthys latos*	*Chenichthys baileyi*
Northern *Characodon*							
Southern *Characodon*	99						
Profundulus labialis	99	99					
Xenotaenia resolanae	98	97	97				
Goodea atripinnis	98	97	97	97			
Empetrichthys latos	97	96	96	96	97		
Chenichthys baileyi	96	95	95	95	96	98	

Table 1. The alignment scores of the sequences compared to one another. A value of 100 means the two sequences are identical. Since all of the northern and southern localities were 100% identical to each other, they were grouped.

Results

A one-nucleotide difference was found between the northern and southern populations of *Characodon* using CLUSTAL W (Appendix A) (Thompson *et al*., 1994). *C. audax* shared the same derived nucleotide as the other northern populations. Phylogenetic analysis of the flanking regions of the microsatellite locus yielded a single, most-parsimonious tree (Figure 8) for the populations of *Characodon* with respect to the outgroups. No variation was detected that supports the validity of *C. lateralis* and *C. audax* as presently recognized. The tree itself (Figure 8) is consistent with the one produced by Howell *et al*. (2008) (Figure 6). Unlike Howell *et al*., the northern populations are not supported as a separate group, which is due to an overall lack of variation in the locus. PAUP returned consistency, rescaled consistency, and homoplasy index values of 1 as a measure of the variation in the locus.

The sequences of *C. audax* (El Toboso in the tree) are indistinguishable from the northern localities of *C. lateralis*. This finding is significant considering that hierarchical variation is present. A previous analysis completed by Elizabeth Hildreth involved a protein-coding gene. She followed a similar procedure to the one utilized here. Her project, however, did not produce any hierarchical variation (unpublished data, 2011). There still has not been enough time for many mutations to accumulate in the genomes (Webb *et al*., 2004).

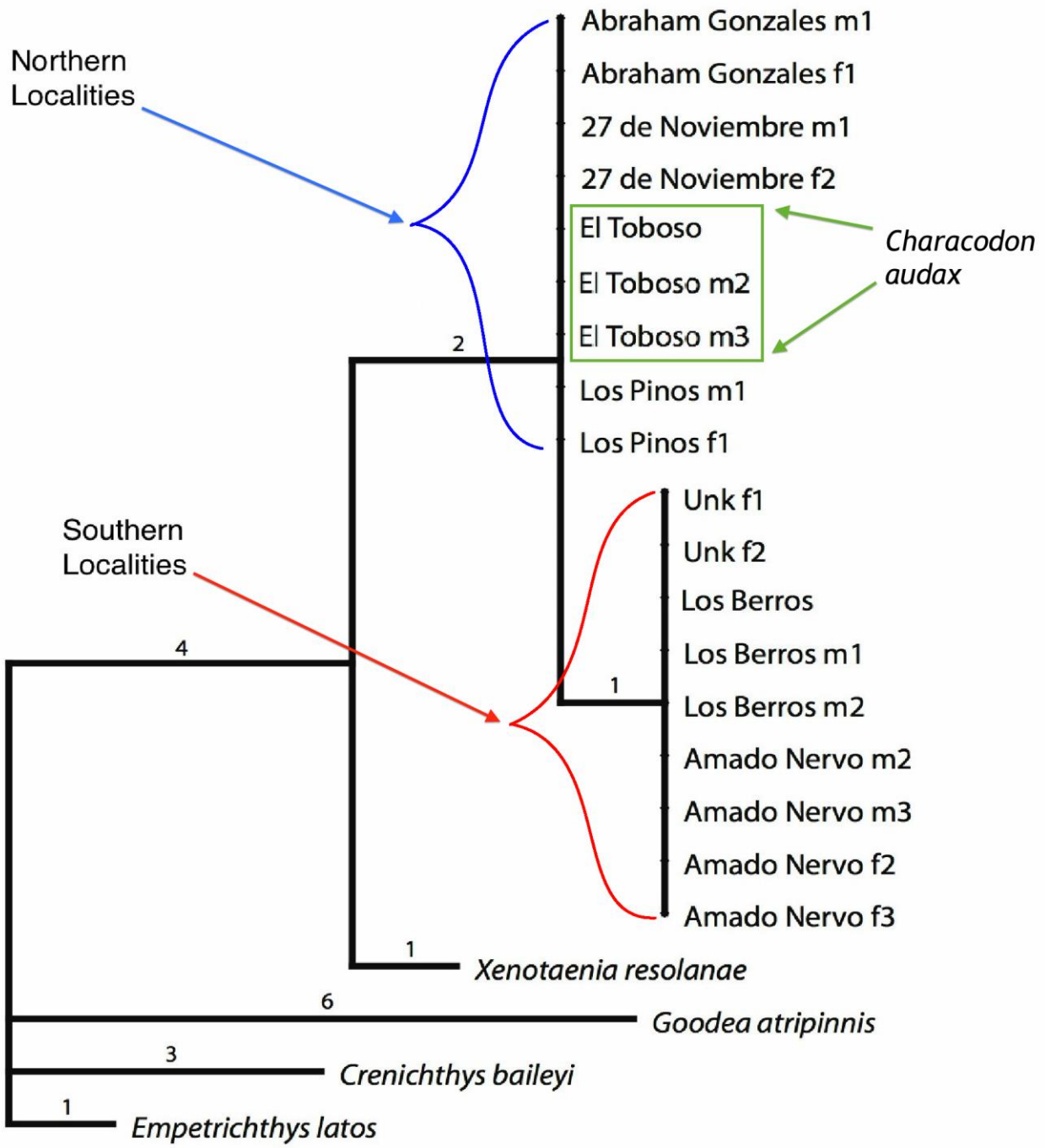

Figure 8. The most-parsimonious phylogenetic tree obtained with DNA from the Zt 1.9 microsatellite locus. El Toboso represents *C. audax*. All other samples are of *C. lateralis*. Each number above the node represents how many nucleotide changes occurred along the branch.

Discussion

Smith and Miller described *C. audax* in 1986. They used what appeared to be a strict rubric of morphological characteristics to differentiate it from *C. lateralis*. Smith and Miller (1986) used physical characteristics such as the distance between the pelvic-fin insertion and the anus, or the presence of a concave dorsal profile. They concluded that because of these differences in morphology, these two fishes warranted species status.

Howell *et al*. (2008), in another attempt to define relationships among these populations, decided to use another type of data. They used 420 nucleotides of DNA from the mitochondrial control region to make a phylogenetic tree and found that *C. lateralis* was likely paraphyletic. *Characodon* appeared to have diverged into northern and southern clades. Because of this finding, Tiedemann (2009) conducted a morphological reanalysis of these fishes. She compared body size and body depth to fin size and length and noted that pelvic fin length was directly related to body depth, not to the identity of the population (*C. audax* or *C. lateralis*). Tiedemann (2009) found similar results with other features, such as the variation in dorsal profiles within *C. audax*. These findings contradicted the conclusions of Smith and Miller (1986).

This study reinforces the findings of Howell *et al.* (2008) and Tiedemann (2009). *C. lateralis* was recovered as paraphyletic in the tree (Figure 8) and appears subdivided into northern and southern clades (but no characters support the monophyly of the northern populations). *C. audax*, again, was no different than the other individuals from the other northern populations. Morphological, mitochondrial, and nuclear data have all suggested that this relationship is questionable, and it is clear that Smith and Miller's description is not valid (Tiedemann, 2009), although currently no conclusion can be drawn until more nuclear data is tested to clarify the relationships of these species.

It is vitally important to identify biologically-meaningful taxonomic units, for it will have a direct impact on the conservation of these fishes. Goodeid species richness has greatly declined in recent years due to anthropogenic factors such as eutrophication, introduction of exotic fishes, and habitat destruction. Conservationists must know how many taxa are still extant, viable, and genetically diverse in order to best protect them (Dominguez-Dominguez *et al*., 2006). If *C. audax* is a valid species, then it must truly be protected, for it only occurs in one small spring (Smith and Miller 1986).

Because this current study was conducted using a small locus with very few characters, more nuclear data is required before making any definitive judgment. It would be helpful to have a longer nuclear sequence with more variation to better clarify *Characodon*. The high indices returned by PAUP (Swofford, 2002) implied that the parsimony-informative character was consistent. Usually, phylogenetic tests of large data sets have complicated histories, so the most parsimonious tree (the one that makes most sense given the data) is chosen. These data produced a single tree because of the low amount of variation overall. This is an effect of small sample size, which is why additional sequences must be found to rigorously test these hypotheses

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Appendix A

The CLUSTAL W alignment of the flanking regions of the Zt1.9 microsatellite locus. The site variable among *Characodon* is shaded.

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   El_Toboso_m3  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
   El_Toboso_m2  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
Abraham_Gonzale  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
Abraham_Gonza_3  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
27_de_Noviembre  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
27_de_Noviemb_5  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
   El_Toboso_EH  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
   Los_Pinos_f1  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
   Los_Pinos_m1  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
Profundulus_lab  GGTAA-GGGG GACCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
  Los_Berros_EH  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
     Unknown_f2  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
     Unknown_f1  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
  Los_Berros_m2  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
  Los_Berros_m1  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
 Amado_Nervo_m3  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
 Amado_Nervo_m2  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
 Amado_Nervo_f3  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
 Amado_Nervo_f2  GGTAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
Xenotaenia_reso  GGTAAAGGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
Goodea_atripinn  GGCAA-GGGG GTCCAACCCG GGACTAGGAA GGTGTGCCTA ATAAAGTGGC
Chrenichthys_ba  GGCAA-GGGG GTCCAACCCG GAACTAGGAA GGTGTACTTA ATAAAGTGGC
Empetrichthys_l  GGCAA-GGGG GTCCAACCCG GAACTAGGAA GGTGTACCTA ATAAAGTGGC

   El_Toboso_m3  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
   El_Toboso_m2  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
Abraham_Gonzale  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
Abraham_Gonza_3  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
27_de_Noviembre  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
27_de_Noviemb_5  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
   El_Toboso_EH  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
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   Los_Pinos_m1  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
Profundulus_lab  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
  Los_Berros_EH  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
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  Los_Berros_m2  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
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 Amado_Nervo_f2  CGTTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGAC
Xenotaenia_reso  CGGTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
Goodea_atripinn  CGGTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA
Chrenichthys_ba  CGGTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACACGACA
Empetrichthys_l  TGGTGAGTGT ACATGTCTGT TTACACTTGC TCTTATCTGA GTACATGACA

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Abraham_Gonzale  CACATCATCA GTCAGTGATA CCAGCTGTGT GTTGAAACCC CTAATCTCTG
Abraham_Gonza_3  CACATCATCA GTCAGTGATA CCAGCTGTGT GTTGAAACCC CTAATCTCTG
27_de_Noviembre  CACATCATCA GTCAGTGATA CCAGCTGTGT GTTGAAACCC CTAATCTCTG
27_de_Noviemb_5  CACATCATCA GTCAGTGATA CCAGCTGTGT GTTGAAACCC CTAATCTCTG
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   Los_Pinos_m1  CACATCATCA GTCAGTGATA CCAGCTGTGT GTTGAAACCC CTAATCTCTG
Profundulus_lab  CACATCATCA GTCAGTGATA CCAGCTGTGT GTTGAAACCC CTAATCTCTG
  Los_Berros_EH  CACATCATCA GTCAGTGATA CTAGCTGTGT GTTGAAACCC CTAATCTCTG
     Unknown_f2  CACATCATCA GTCAGTGATA CTAGCTGTGT GTTGAAACCC CTAATCTCTG
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Xenotaenia_reso  CACATCATCA GTCAGTGATA CCAGCTGTGT GTTGAAACCC CTAATCTCTG
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Abraham_Gonzale  CATGTTTATC TACATATTGC TTTACCCGGC GATGTTGGCC ATGGAGCTGA
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27_de_Noviemb_5  CATGTTTATC TACATATTGC TTTACCCGGC GATGTTGGCC ATGGAGCTGA
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  Los_Berros_m2  CATGTTTATC TACATATTGC TTTACCCGGC GATGTTGGCC ATGGAGCTGA
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Chrenichthys_ba  CATGTTTATC TACATGTTGC TTTACCCGGC GATGTTGGCC ATGGAGCTGA
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Abraham_Gonzale  GTGCATCATA TCCTTGTGCG ATTGTAACTC TGG-CACC
Abraham_Gonza_3  GTGCATCATA TCCTTGTGCG ATTGTAACTC TGG-CACC
27_de_Noviembre  GTGCATCATA TCCTTGTGCG ATTGTAACTC TGG-CACC
27_de_Noviemb_5  GTGCATCATA TCCTTGTGCG ATTGTAACTC TGG-CACC
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Profundulus_lab  GTGCATCATA TCCTTGTGCG ATTGTAACTC TGG-CACC
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Xenotaenia_reso  GTGCATCATA TCCTTGTGCG ATTGTAACTC TGG-CACC
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